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Combination In Vitro and in Silico Methodology for Risk Assessment of Long QT Type 1 Patients

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There is a long history of simulating the effects of channelopathies on the cardiac action potential (AP) in diseases like Long QT (LQT) Syndrome. While an important proof of concept, studies have been limited because the number of mutants considered was small and correlation with the phenotype was anecdotal. This study seeks to address such limitations by including 17 Long QT type 1 (LQT 1) mutations for which both *in vitro* characterizations have been performed and detailed clinical outcome data are known. The IKs channel (KCNQ1 and KCNE1 subunits) were expressed in both *Xenopus* oocytes and HEK-293T cells, and G_{max}, V_{half} and the τ of activation and deactivation were measured. The properties were then incorporated into a humanized version of the Flaim-Giles-McCulloch reconstruction of canine cardiomyocytes. The model also included beta-adrenergic stimulation that is known to modulate IKs and is thought to contribute to exercise-induced sudden death in some LQT patients. Both single cell and 1-D cable models were investigated. The simulated QT prolongation in the cable model correlated well with the QTc in patients carrying the 17 mutations studied. The cable model including beta-adrenergic stimulation showed early afterdepolarizations (EADs) and T-wave alternans for mutants. The presence of T-wave alternans correlated with cardiac risk for these patients. Correlation of electrophysiology and clinical phenotype showed that slow activation rates for LQT1 mutations are risk factors for these patients independent from QT prolongation. Incorporation of slow IKs activation into the model produced a more severe to response to β-adrenergic-mediated EADs as compared to responses with reduced IKs conductance only. Our results suggest that cellular electrophysiology in combination with 1-D cable models is a good methodology to predict cardiac risk associated with Long QT1 mutations.

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SERCA2 Knockout Mice Exhibit Impaired Control of Ca^{2+} Current but not Ventricular Arrhythmias

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Impaired Ca^{2+} handling by the sarcoplasmic reticulum (SR) and Ca^{2+}-dependent arrhythmias are hallmark features of human heart failure. We investigated the control of L-type Ca^{2+} current ($I_{Ca,\ L}$) when SR function is reduced and the consequences for arrhythmogenesis. Experiments were performed on cardiomyocytes isolated from conditional SERCA2 KO mice (KO) which had developed heart failure 7 weeks following gene disruption. $SERCA2^{flox/flox}$ (FF) mice served as controls. SR Ca^{2+} content was reduced to 4% ($P<0.05$) of FF values in KO cardiomyocytes, and SR Ca^{2+} release did not occur on a beat-to-beat basis. Marked up-regulation of the L-type Ca^{2+} channel in KO (α_{1C} subunit= 178% FF, α_2/δ_1= 147% FF, $P<0.05$) was accompanied by a 40% increase in peak $I_{Ca,\ L}$ ($P<0.05$). Loss of SR function resulted in slower Ca^{2+} current inactivation, prolonged duration of current activation, and loss of frequency-dependent facilitation. The larger magnitude and prolonged $I_{Ca,L}$ in KO resulted in AP prolongation, which was not observed in the presence of nifedipine or upon removal of extracellular Ca^{2+}. AP prolongation was associated with prolonged QT intervals corrected for heart rate in KO mice compared to FF (5.63 ms vs 4.90 ms, $P<0.05$). While AP prolongation is expected to be arrhythmogenic, incidence of early afterdepolarizations in KO cardiomyocytes was not increased (FF= 2/13 cells, KO= 0/10 cells, P=NS). Telemetric ECG surveillance during pharmacological stress also revealed a similar incidence of ventricular arrhythmias in FF and KO mice. In conclusion, loss of SR function results in greater L-type Ca^{2+} entry, loss of Ca^{2+}-dependent inactivation, and prolonged APs and QT interval. While such alterations would be expected to pro-arrhythmic in larger species, the relatively brief AP in failing mice may preclude occurrence of early after-depolarizations.

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Non-Genomic Effects of 17beta-Estradiol on Cardiomyocytes

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Pre-menopausal women have a reduced risk from ischemic heart disease compared to men; this protection is lost after ovariectomy or menopause. Estrogen has been found to confer protection in whole-heart studies in animal models. ATP-sensitive K^+ (K_{ATP}) channels act as metabolic sensors, opening when cellular energy levels fall and are thought to play a role in cardioprotection. We investigated whether the cardiac K_{ATP} currents play a role in the cardioprotective effects of 17β-estradiol in isolated cardiomyocytes.

Ventricular myocytes were isolated from adult male Wistar rats by enzymatic digestion. The ability of myocytes to recover contractile function after simulated ischaemia/reperfusion injury was calculated by video microscopy. Contracting myocytes (1Hz) were continuously superfused with Tyrode solution, then subjected to 7 minutes of similated ischaemia by perfusion with a metabolic inhibition solution, comprising substrate-free Tyrode with cyanide (2mM) and idoacetic acid (1mM), followed by 10 minute reperfusion with Tyrode to simulate reperfusion. A 5 minute pretreatment of 17β-estradiol (500nM) significantly increased contractile recovery; control 30% n=100, 17β-estradiol 68% n=185, DPN (ERβ agonist) 53% n= 111.

Using the whole-cell patch-clamp mode we investigated the effect of estrogen on K_{ATP} channel activity. P1075 (10μM) elicited a K_{ATP} current which was completely blocked by bath application of estradiol (500nM). Both the classical estrogen receptors; ERα and ERβ have been implicated in several rapid effects of 17β-estradiol. DPN is a specific agonist of ERβ and application of DPN (500nM) following opening of K_{ATP} by P1075 (10μM) also resulted in 100% decrease in K_{ATP} current.

We show that rapid estrogen signalling has a cardioprotective effect in isolated ventricular myocytes. Application of estradiol resulted in complete loss of K_{ATP} current recorded in cardiomyocytes; therefore questioning the role of K_{ATP} in estrogen-dependent cardioprotection.

This work was supported by funding by The British Heart Foundation.

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Extracellular Proton Modulation of Peak and Late Sodium Current in the Canine Left Ventricle

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Background: Cardiac ischemia produces a reduction in excitability in ventricular tissue. Acidosis (one component of ischemia) affects a number of ion currents. We examined the effects of extracellular acidosis on Na^+ current in canine ventricular cells. **Methods:** Epicardial (Epi) and endocardial (Endo) myocytes were isolated from the left ventricle. Voltage clamp methods were used to record I_{Na}. Peak I_{Na} was recorded in low external Na^+ to ensure voltage control. Late I_{Na} was recorded in full external Na^+ and defined as the TTX-sensitive current. **Results:** Action potential recordings from left ventricular wedges exposed to pH=6.6 showed a widening of the QRS complex indicating slowing of transmural conduction. In myocytes, exposure to acidic conditions caused a $17.3 \pm 0.9\%$ reduction in upstroke velocity at pH=6.6 versus 7.4. Patch clamp analysis of fast I_{Na} showed current density was similar in Epi and Endo cells at normal pH (68.1 ± 7.0 pA/pF versus 63.2 ± 7.1 pA/pF respectively at −35 mV). Extracellular acidosis reduced fast I_{Na} magnitude by 22.7% in Epi cells and 23.1% in Endo cells. In addition, a slowing of the decay (τ) of fast I_{Na} was observed at pH=6.6. Acidosis did not affect steady state inactivation of I_{Na} or recovery from inactivation. Analysis of late I_{Na} during a 500 ms pulse showed acidosis reduced late I_{Na} at 250 and 500ms into the pulse but no reduction was observed 50ms into the pulse. **Conclusions:** We demonstrate that acidosis reduces the size of peak I_{Na} and slows the decay without affecting Na^+ channel availability or recovery. Acidosis also reduced the TTX-sensitive late I_{Na}. The reduction in peak and late I_{Na} observed during acidosis may contribute to the depression in cardiac excitability observed under ischemia.

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Normalizing Action-Potential Morphology in Long-Term Cultures of Adult Guinea-Pig Ventricular Cardiomyocytes by Ca_VBETA Expression

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Adult guinea-pig ventricular myocytes (aGPVMs) exhibit enduring action-potential plateaus similar to those in humans, making this system advantageous for investigating human phase 2 phenomena. Long-term cultures of aGPVMs (Canadian J Physiol Pharmacol**7**:740) further offer the promise of genetic manipulation by somatic gene transfer,